

CLAIMS

What is claimed is:

1. An assay for assessing the extent to which a cell is metabolically active, which comprises: measuring an amount of light emitted by a cell comprising an expressible heterologous *lux(CDABE)* gene complex under the control of a constitutive promoter; changing the environment of said cell; and comparing the amount light emitted by said cell prior to said change with the amount of light emitted by said cell after said change; where an increase in said amount of said light emitted after said change means that said cell is more metabolically active after said change; and where a decrease in said amount of said light emitted after said change means that said cell is less metabolically active after said change; provided that, said cell produces an amount of FMNH<sub>2</sub> sufficient for the light producing reaction when said cell is metabolically active.
2. The assay as defined in claim 1 wherein said *lux(CDABE)* gene complex is isolated from *Xenorhabdus luminescens*.
3. The assay as defined in claim 1 wherein said cell is in a medium, and said change comprises the addition of a test agent to said medium.
4. The assay as defined in claim 3 wherein said medium further comprises an exogenous metabolic activation system for said test agent.
5. The assay as defined in claim 4 wherein said cell is selected from the group consisting of *Salmonella typhimurium* TA1535/*lux* (comprising pBRTN/*lux*1) (UC25447), *Salmonella typhimurium* TA100/*lux* (comprising pBRTN/*lux*2) (UC25448), *Salmonella typhimurium* TA98/*lux* (comprising pTN/*lux*2) (UC25449), said medium is substantially histidine-free, said exogenous metabolic activation system is an S-9 preparation, and said increase after said addition of said test means that said test agent reversed the histidine gene mutation of said cell.
6. The assay as defined in claim 4 wherein said cell is *Escherichia coli* WP2/*lux* (comprising pBRTN/*lux*1) (UC25452), said medium is substantially tryptophan-free, said exogenous metabolic activation system is an S-9 preparation,

and said increase after said addition of said test means that said test agent reversed  
5 the tryptophan gene mutation of said cell.

7. The assay as defined in claim 4 wherein cell is *Salmonella typhimurium* TA2220lux (comprising pBRTN/luxAM1) (UC25450) or *Salmonella typhimurium* TA2211lux (comprising pBRTN/luxAMmuc631) (UC25451), said medium comprises ampicillin, said exogenous metabolic activation system is an S-9  
5 preparation, and said increase after said addition of said test agent means that said test agent reversed the  $\beta$ -lactamase gene mutation of said cell.

8. A cell comprising an expressible heterologous *lux(CDABE)* gene complex and a substantially reversible point mutation. *oh*

9. A cell as defined in claim 8 wherein said point mutation is in a histidine gene. *oh*

10. A cell as defined in claim 9 wherein said cell is selected from the group consisting of: *Salmonella typhimurium* TA1535lux (UC25447), *Salmonella typhimurium* TA100lux (UC25448), and *Salmonella typhimurium* TA98lux (UC25449). *oh*

11. A cell as defined in claim 8 wherein said point mutation is in a tryptophan gene. *oh*

12. The cell as defined in claim 11 wherein said cell is *Escherichia coli* WP2lux (UC25452). *oh*

13. A cell as defined in claim 8 wherein said point mutation is in a  $\beta$ -lactamase gene *oh*

14. A cell as defined in claim 13 wherein said point mutation in said  $\beta$ -lactamase is in the active site serine codon. *oh*

15. A cell as defined in claim 14 selected from *Salmonella typhimurium* TA2220lux (UC25450) and *Salmonella typhimurium* TA2211lux (UC25451). *oh*

16. A bioluminescent reverse mutagenicity assay, which comprises: bioluminescent reverse mutagenicity assays, which comprise: contacting a bacterial cell with a test agent and an exogenous metabolic activation system, where said cell comprises an expressible heterologous *lux(CDABE)* gene complex (or operon) and a  
5 reversible point mutation in a gene which in a non-mutated form encodes a polypeptide whose functioning is critical for the cell to be metabolically active in a

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selective medium; measuring an amount of light emitted from said cell; and comparing said amount of said light emitted by said cell exposed to said test agent and said exogenous metabolic activation system with substantially the same cell contacted with an exogenous metabolic activation system in the absence of said test agent; where an amount of emitted light is detected in said cell contacted with said test agent and said exogenous metabolic activation system, and substantially no amount of emitted light is detected in said cell exposed to said exogenous metabolic activation system in the absence of said test agent, means that said test agent is a mutagen; provided that, said cell produces an amount of FMNH<sub>2</sub> sufficient for the light producing reaction when said cell is metabolically active.

17. An assay for assessing the extent to which a cell is metabolically active, comprising: measuring an amount of light emitted by a cell comprising an expressible heterologous *luc* gene under the control of a constitutive promoter, in the presence of an amount of a luciferin; changing the environment of said cell; and comparing the amount of light emitted by said cell prior to said change with the amount of light emitted by said cell after said change; where an increase in said amount of said light emitted after said change means that said cell is more metabolically active after said change; and where a decrease in said amount of said light emitted after said change means that said cell is less metabolically active after said change; provided that said cell produces an amount of ATP sufficient for the light producing reaction when said cell is metabolically active.

18. The assay as defined in claim 17 wherein said cell is in a medium, and said change comprises the addition of a test agent to said medium.

19. The assay as defined in claim 18 wherein said medium further comprises an exogenous metabolic activation system for said test agent.

20. The assay as defined in claim 18 wherein said cell also comprises a reversible point mutation in a gene, where a non-mutated form of said gene encodes a polypeptide critical for functioning of said cell in a selective medium.

21. The assay as defined in claim 19 wherein said cell also comprises a reversible point mutation in a gene where a non-mutated gene provides a component critical for functioning of said cell in a selective medium.

22. The assay as defined in claim 21 wherein said medium is substantially free of said critical component provided by said non-mutated gene, said exogenous metabolic activation system is an S-9 preparation, and said increase after said addition of said test means that said test agent reversed the point mutation in said gene of said cell.

23. A method for determining the structural and chemical characteristics of compounds that confer mutagenicity to said compounds, which comprises: contacting substantially identical bacterial cells with an amount of each compound of a compound library and an amount of an exogenous metabolic activation system substantially sufficient to activate said compound, where said cells each comprise an expressible heterologous *lux(CDABE)* gene complex (or operon) and a reversible point mutation in a gene which in a non-mutated form encodes a polypeptide whose functioning is critical for the cell to be metabolically active in a selective medium; measuring an amount of light emitted from each of said cells; and comparing said amount of said light emitted by each of said cells exposed to said amount of said compound of said compound library and said exogenous metabolic activation system with substantially the same cell contacted with an exogenous metabolic activation system in the absence of said amount of said compound of said compound library; where an amount of emitted light is detected in said cell contacted with said amount of said compound of said compound library and said exogenous metabolic activation system, and substantially no amount of emitted light is detected in said cell exposed to said exogenous metabolic activation system in the absence of said amount of a compound of said compound library, means that said compound of said compound library is a mutagen; and comparing the chemical and structural characteristics of each of said compounds of said library that caused light to be emitted to identify common characteristics; provided that, said cell produces an amount of FMNH<sub>2</sub> sufficient for the light producing reaction when said cell is metabolically active.

24. A method for determining the structural and chemical characteristics of compounds that confer mutagenicity to said compounds, which comprises: which comprise: contacting substantially identical mammalian cells with an amount of each compound of a compound library, and an amount of an exogenous metabolic activation system substantially sufficient to activate said compound, where said cells

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each comprise an expressible heterologous *luc* gene and a reversible point mutation in a gene which in a non-mutated form encodes a polypeptide whose functioning is critical for the cell to be metabolically active in a selective medium, in the presence of a substrate for said polypeptide; measuring an amount of light emitted from each of

10 said cells; and comparing said amount of said light emitted by each of said cells exposed to said amount of said compound of said compound library and said exogenous metabolic activation system with substantially the same cell contacted with an exogenous metabolic activation system in the absence of said amount of said compound of said compound library; where an amount of emitted light is detected in

15 said cell contacted with said amount of said compound of said compound library and said exogenous metabolic activation system, and substantially no amount of emitted light is detected in said cell exposed to said exogenous metabolic activation system in the absence of said amount of said compound of said compound library, means that said compound of said compound library is a mutagen; and comparing the chemical

20 and structural characteristics of each of said compounds of said library that caused light to be emitted to identify common characteristics; provided that, said cell produces an amount of ATP sufficient for the light producing reaction when said cell is metabolically active.

FOOTNOTES